

CHIR0312.ST25  
SEQUENCE LISTING

<110> Pizza, Mariagrazia  
Fontana, Maria Rita  
Giannelli, Valentina  
Rappuoli, Rina

<120> Immunogenic Detoxified Mutants Of Cholera Toxin

<130> CHIR0312

<150> 08/981,208

<151> 1997-12-22

<150> 9513371.6

<151> 1995-06-30

<160> 8

<170> PatentIn version 3.0

<210> 1

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<212> DNA

<213> Artificial Sequence

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<221> misc\_feature

<223> Primer

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<210> 5  
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 <212> PRT  
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Asn Asp Phe Phe Arg Ala Asp Ser Arg Thr Pro Asp Glu Ile Arg Gln  
 1 5 10 15  
 Ala Gly Gly Leu Leu Pro Arg Gly Gln Gln Glu Ala Tyr Glu Arg Gly  
 20 25 30  
 Thr Pro Ile Asn Ile Asn Leu Tyr Glu His Ala Arg Gly Thr Val Thr  
 35 40 45  
 Gly Asn Thr Arg His Asn Asp Gly Tyr Val Ser Thr Thr Val Thr Leu  
 50 55 60  
 Arg Gln Ala His Leu Ile Gly Gln Asn Ile Leu Gly Ser His Asn Glu  
 65 70 75 80  
 Tyr Tyr Ile Tyr Val Val Ala Pro Ala Pro Asn Leu Phe Asp Val Asn  
 85 90 95  
 Gly Val Leu Gly Arg Tyr Ser Pro Tyr Pro Ser Glu Asn Glu Phe Ala  
 100 105 110  
 Ala Leu Gly Gly Ile Pro Leu Ser Gln Ile Ile Gly Trp Tyr Arg Val  
 115 120 125  
 Ser Phe Gly Ala Leu Glu Gly Gly Met Gln Arg Asn Arg Asp Tyr Arg  
 130 135 140  
 Gly Asp Leu Phe Ser Gly Leu Thr Val Ala Pro Asn Ala Asp Gly Tyr  
 145 150 155 160  
 Gln Leu Ala Gly Phe Pro Ser Asn Phe Pro Ala Trp Arg Glu Met Pro  
 165 170 175  
 Trp Ser Thr Phe Ala Pro Glu Gln Cys Val Pro Asn Asn Lys Glu Phe  
 180 185 190  
 Lys Ser Gly Val Cys Ile Ser Ala Thr Asn Val Leu Gly Lys Tyr Asp  
 195 200 205  
 Leu Met Asn Phe Lys Lys Leu Leu Lys Arg Arg Leu Ala Leu Thr Phe  
 210 215 220  
 Phe Met Ser Asp Asp Asp Phe Thr Gly Val His Gly Glu Lys Asp Glu  
 225 230 235 240

Leu

## CHIR0312.ST25

<210> 6  
 <211> 236  
 <212> PRT  
 <213> E. coli

<400> 6

Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
 1 5 10 15  
 Lys Gln Phe Arg Ser Leu Met Pro Arg Gly Ser Glu Tyr Phe Asp Arg  
 20 25 30  
 Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr Gln  
 35 40 45  
 Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile Ser  
 50 55 60  
 Leu Arg Ser Ala His Leu Val Gly Gln Tyr Ile Leu Ser Gly His Ser  
 65 70 75 80  
 Leu Thr Ile Tyr Ile Val Ile Ala Asn Met Phe Asn Val Asn Asp Val  
 85 90 95  
 Ile Ser Ala Tyr Ser Pro His Pro Asp Glu Gln Glu Val Ser Ala Leu  
 100 105 110  
 Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr Arg Val His Phe  
 115 120 125  
 Gly Val Leu Asp Glu Gln Leu His Arg Asn Arg Gly Tyr Arg Asp Arg  
 130 135 140  
 Tyr Tyr Ser Asn Leu Asp Ile Ala Pro Ala Ala Asp Gly Tyr Gly Leu  
 145 150 155 160  
 Ala Gly Phe Pro Pro Glu His Arg Ala Trp Arg Glu Glu Pro Trp Ile  
 165 170 175  
 His His Ala Pro Pro Gly Cys Gly Asn Ala Pro Arg Ser Ser Met Ser  
 180 185 190  
 Asn Thr Cys Asp Glu Lys Thr Gln Ser Leu Gly Val Lys Phe Leu Asp  
 195 200 205  
 Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser Gly Tyr Gln Ser  
 210 215 220  
 Asp Ile Asp Thr His Asn Arg Ile Lys Asp Glu Leu  
 225 230 235

<210> 7  
 <211> 240  
 <212> PRT  
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<400> 7

Asn Gly Asp Arg Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
 1 5 10 15  
 Lys Arg Ser Gly Gly Leu Met Pro Arg Gly His Asn Glu Tyr Phe Asp

20

25

30

Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr  
 35 40 45

Gln Thr Gly Phe Val Arg Tyr Asp Asp Gly Tyr Val Ser Thr Ser Leu  
 50 55 60

Ser Leu Arg Ser Ala His Leu Ala Gly Gln Ser Ile Leu Ser Gly His  
 65 70 75 80

Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  
 85 90 95

Val Asn Asp Val Leu Gly Val Tyr Ser Pro His Pro Tyr Glu Gln Glu  
 100 105 110

Val Ser Ala Leu Gly Gly Ile Pro Tyr Ser Gln Ile Tyr Gly Trp Tyr  
 115 120 125

Arg Val Asn Phe Gly Val Ile Asp Glu Arg Leu His Arg Asn Arg Glu  
 130 135 140

Tyr Arg Asp Arg Tyr Tyr Arg Asn Leu Asn Ile Ala Pro Ala Glu Asp  
 145 150 155 160

Gly Tyr Arg Leu Ala Gly Phe Pro Pro Asp His Gln Ala Trp Arg Glu  
 165 170 175

Glu Pro Trp Ile His His Ala Pro Gln Gly Cys Gly Asp Ser Ser Arg  
 180 185 190

Thr Ile Thr Gly Asp Thr Cys Asn Glu Glu Thr Gln Asn Leu Ser Thr  
 195 200 205

Ile Tyr Leu Arg Glu Tyr Gln Ser Lys Val Lys Arg Gln Ile Phe Ser  
 210 215 220

Asp Tyr Gln Ser Glu Val Asp Ile Tyr Asn Arg Ile Arg Asp Glu Leu  
 225 230 235 240

<210> 8  
 <211> 240  
 <212> PRT  
 <213> Vibrio cholerae

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Asn Asp Asp Lys Leu Tyr Arg Ala Asp Ser Arg Pro Pro Asp Glu Ile  
 1 5 10 15

Lys Gln Ser Gly Gly Leu Met Pro Arg Gly Gln Ser Glu Tyr Phe Asp  
 20 25 30

Arg Gly Thr Gln Met Asn Ile Asn Leu Tyr Asp His Ala Arg Gly Thr  
 35 40 45

Gln Thr Gly Phe Val Arg His Asp Asp Gly Tyr Val Ser Thr Ser Ile  
 50 55 60

Ser Leu Arg Ser Ala His Leu Val Gly Gln Thr Ile Leu Ser Gly His  
 65 70 75 80

Ser Thr Tyr Tyr Ile Tyr Val Ile Ala Thr Ala Pro Asn Met Phe Asn  
 85 90 95

## CHIR0312.ST25

Val	Asn	Asp	Val	Leu	Gly	Ala	Tyr	Ser	Pro	His	Pro	Asp	Glu	Gln	Glu	100	105	110
Val	Ser	Ala	Leu	Gly	Gly	Ile	Pro	Tyr	Ser	Gln	Ile	Tyr	Gly	Trp	Tyr	115	120	125
Arg	Val	His	Phe	Gly	Val	Leu	Asp	Glu	Gln	Leu	His	Arg	Asn	Arg	Gly	130	135	140
Tyr	Arg	Asp	Arg	Tyr	Tyr	Ser	Asn	Leu	Asp	Ile	Ala	Pro	Ala	Ala	Asp	145	150	155
Gly	Tyr	Gly	Leu	Ala	Gly	Phe	Pro	Pro	Glu	His	Arg	Ala	Trp	Arg	Glu	165	170	175
Glu	Pro	Trp	Ile	His	His	Ala	Pro	Pro	Gly	Cys	Gly	Asn	Ala	Pro	Arg	180	185	190
Ser	Ser	Met	Ser	Asn	Thr	Cys	Asp	Glu	Lys	Thr	Gln	Ser	Leu	Gly	Val	195	200	205
Lys	Phe	Leu	Asp	Glu	Tyr	Gln	Ser	Lys	Val	Lys	Arg	Gln	Ile	Phe	Ser	210	215	220
Gly	Tyr	Gln	Ser	Asp	Ile	Asp	Thr	His	Asn	Arg	Ile	Lys	Asp	Glu	Leu	225	230	235
																		240